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SPECIFIC ANTIBODIES IN SCARLET FEVER.*†

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The demonstration of specific antibodies in scarlet fever has been limited chiefly to those for streptococci. The numerous investigations which deal with this subject, carried out with the object of determining the rôle which the streptococcus plays in the etiology of scarlet fever, may here be reviewed in brief, before we proceed with the report of our own work.

ANTIBODIES FOR STREPTOCOCCI.

I. *Agglutinins.*—Grünbaum[1] seems to have been the first to report on the agglutination of streptococci of scarlet fever by the serums of scarlet-fever patients. He simply states that he was able to isolate a coccus from a case of scarlet fever which agglutinated with scarlet-fever serum.

Baginsky and Sommerfeld[2] examined the serums of scarlet-fever patients in the third to the sixth week of the disease, for specific agglutinins of streptococci, which were cultivated from cases of scarlet fever. Their results were absolutely negative. At about the same time Moser and von Pirquet[3] found that in 52 per cent of the cases examined the serum caused marked agglutination of scarlatinal streptococci. The serum from cases other than scarlet fever gave the reaction in a much smaller percentage and only on higher dilution.

Salge and Hasenknopf[4] made a comprehensive study of this question. They found that emulsions of streptococci of scarlet fever were markedly agglutinated by the serum of scarlatina in a dilution as high as 1:500, whereas the reaction failed with streptococci from other sources, even with a lesser dilution of the serum. This specific property of the serum vanishes toward the end of convalescence.

Dopter[5] denies the specific relation of scarlet-fever serum toward homologous streptococci. In his hands streptococci of various origin were agglutinated by the serum of scarlet-fever patients and indeed in some cases the serum did not react with streptococci from scarlet-fever cases. Dopter reaches the conclusion that the streptococcus is not the specific agent of the disease.

Detot,[6] and Detot and Boucart[7] arrived at similar results. Since the agglutination is not specific they reject its application as a diagnostic means and deny the rôle of the streptococcus in the etiology of scarlet fever.

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* Received for publication August 31, 1911.

† This work was aided by a grant from the American Medical Association.

[1] *Science Progress*, 1897, 1, p. 616; *Lancet*, 1897, 1, p. 451.

[2] *Arch. f. Kinderh.*, 1902, 33, p. 1.

[3] *Centralbl. f. Bact.* I, Orig., 1904, 36, p. 692.

[4] *Jahrb. f. Kinderh.*, 1903, 58, p. 218.

[5] *Compt. rend. Soc. de biol.*, 1904, 56, p. 787.

[6] *Ibid.*, 57, p. 44.

[7] *Rev. mens. de malad. de l'enfance*, 1905, 23, p. 64.

In the same year there appeared an important article by Weaver[1] on the agglutination of streptococci by scarlet-fever serum. He too comes to the conclusion that the agglutinins in the serum are not specific; that in the serum of patients with erysipelas, pneumonia, and even other infections of non-streptogenous nature, agglutinins for scarlet-fever streptococci may be present. Weaver points out further that the discordant results of the different investigators may be due to the varying composition and reaction of the culture medium.

Jogiches[2] found that the serum of scarlatina agglutinated streptococci of the most different origin even in dilution of 1:500 and 1:600; the reaction appears in about the fifth to the sixth week of the disease, but fails entirely in the beginning of the disease. There exists no constant difference in the intensity of the reaction toward the various strains of streptococci.

Rossiwal and Schick[3] found several streptococci, biologically different, in one case of scarlet fever, which gave various high agglutination figures with Moser's antistreptococcus serum.

Rüdiger,[4] on the other hand, found that the serum of a sheep immunized against a streptococcus isolated from the throat of scarlet-fever cases contained specific agglutinins for this strain only, and that the *Streptococcus viridans* was not agglutinated.

II. *Bacteriolysins* —Weaver and Rüdiger[5] have shown that neither normal nor scarlet-fever serum contains lysins for streptococci.

III. *Phagocytosis and Opsonins.*—The importance of phagocytosis in streptococcus infections was demonstrated by Metchnikoff in the case of erysipelas. Denys and Leclef[6] showed that immune serum promotes the phagocytosis of streptococci, though the term opsonin was not then used. The important contributions of Denys and Marchand, Bordet, Hektoen, Neufeld and Rimpau, and Rüdiger cannot be considered here, as they deal mostly with the mechanism of experimental streptococcus infections, while we limit ourselves here to the production of antibodies and their demonstration in scarlet fever.

Ruth Tunnicliff[7] found that in the beginning of the disease the streptococco-opsonic index is below normal in the majority of cases. As the acute symptoms subside, the index rises above normal, to which it returns, at times, quite abruptly; and in uncomplicated cases it usually remains normal during convalescence. Definite localized streptococcus complications are inaugurated by a depression in the streptococco-opsonic index, which rises again as improvement sets in. These variations of the opsonic index are limited to the streptococcus and are absent with respect to pneumococcus, staphylococcus, and pseudodiphtheria bacillus; hence "the streptococco-opsonin in scarlet fever is a specific opsonin."

Banks[8] arrives at similar results: In cases of scarlet fever running a normal course the opsonic power is decreased during the early febrile period and rises to normal or above normal with the defervescence. In fatal cases the opsonic power is markedly subnormal. Complications alter the curve; the opsonic power is decreased at the onset and during the earlier period of albuminuria, severe nephritis, and secondary adenitis. Banks believes in a definite relationship of the *Streptococcus scarlatinae* to the disease, yet there is no striking difference in the results obtained with different varieties

[1] *Jour. Infect. Dis.*, 1904, 1, p. 91.

[2] *Centralbl. f. Bact.*, I, Orig., 1904, 36, p. 692.

[3] *Wien. klin. Wchnschr.*, 1905, 18, p. 3.

[4] *Jour. Infect. Dis.*, 1906, 3, p. 755.

[5] *Trans. Chic. Path. Soc.*, 1903, 5, p. 285.

[6] *La cellule*, 1895, 11, p. 175.

[7] *Jour. Infect. Dis.*, 1907, 3, p. 304.

[8] *Jour. of Path. and Bact.*, 1907, 12, p. 113.

of streptococci. These findings of Tunnicliff and of Banks were confirmed later by Jochmann and Michaelis.[1]

IV. *Antitoxins.*—All investigators, who have occupied themselves with the proof of antitoxic substances in the serum of scarlatinal patients, have arrived at negative results. Neither Besredka, nor Neufeld, nor Moser and Schwoner were able to demonstrate antistreptocolysins (antihemotoxins) in the serum.

V. *Bordet's Antibodies for Streptococci.*—Besredka and Dopter[2] examined the serum of scarlatinal patients for complement-deviating antibodies for streptococci. Besredka himself previously had found with this method antibodies in streptococcus-immune serums. As antigen they used streptococci cultivated from the throat and blood of scarlet-fever patients. As they were not able to find a specific "fixateur" in a single case they considered themselves justified in concluding that the streptococcus plays no specific part in scarlet fever, but has the rôle of a secondary participant. Foix and Mallein[3] repeated these experiments with more positive results. They used a polyvalent antigen of streptococci which had been cultivated from numerous cases of scarlatinal angina. Though the cases examined are small in number the results obtained are interesting. With twelve serums of scarlet-fever cases they obtained deviation of the complement ten times. Controls made with serum of other streptococcus infections, as erysipelas and puerperal sepsis showed complete hemolysis. The antibodies were present as early as the fourth, and as late as the 38th, day.

Livierato[4] examined 18 scarlatinal serums using as antigen extracts of streptococci, staphylococci, and pneumococci, typhus, colon, and influenza bacilli. Complete inhibition of hemolysis was obtained by the streptococcus extract only, and this in every case, though the streptococci used were not cultivated from cases of scarlet fever. Livierato considers these findings an important contribution toward the proof of the streptocogenous nature of scarlet fever.

H. E. Eggers[5] obtained "rather rough positive reactions in a certain proportion of cases, but could not obtain the reaction in all cases." He points out the important fact that broth cultures of streptococci used as antigen may be a source of error in performing the reaction, as sterile broth in itself possesses complement-fixing (antihemolytic?) properties.

Schleissner[6] used suspensions of streptococci from scarlatina, erysipelas, puerperal sepsis, and panophthalmia as antigen. The serum of scarlatina, especially from the seventh to the 35th day, reacted positively with scarlatinal streptococci, whereas control serums from other affections never gave a positive reaction. With erysipelas streptococci, scarlet-fever serums never gave deviation, though some reacted positively with the streptococci of puerperal sepsis and panophthalmia. This latter reaction, however, was quantitatively weaker than the reaction with the scarlatinal streptococci.

From all these investigations it results clearly that the serum of scarlatina patients contains antibodies for the streptococcus. This speaks undeniably for the biological relation of this microorganism to scarlet fever. The existence of a specific scarlatinal streptococcus, however, is not demonstrated by this work, and no light has been shed on the primary etiology of the disease. The very presence of antibodies cannot solve the problem whether the streptococcus is the primary agent of the disease or a secondary invader. And even if it had been shown that these reactions were obtainable only with scarlatinal serum, and streptococci cultivated from scarlatina, the question of the etiology of the disease would still be unsolved. In this connection the work of DeWael and Sugg[1] may be recalled. They found that the streptococci, constantly present in smallpox, are specifically and exclusively agglutinated by the serum and other serous liquids of variola patients and convalescents (dilution 1:400–1:800), but not by any other immune streptococcus serum. Yet one would hardly conclude from this that the streptococcus is the etiologic agent of variola.

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[1] *Berl. klin. Wchnschr.*, 1910, 47, p. 921.

[2] *Ann. de l'Inst. Pasteur*, 1904, 18, p. 373.

[3] *Presse méd.*, 1907, 15, p. 777.

[4] *Centralbl. f. Bact.*, I, 54, p. 422.

[5] *Chic. Path. Soc.*, 1908, 7, p. 166.

[6] *Folia serologica*, 1909, 3, p. 271.

It therefore seemed appropriate to use those methods for the examination of antibodies which have proved valuable in other diseases of unknown origin, and to exclude as far as possible the streptococcus and its antibodies in the arrangement of the experiment.

SPECIFIC ANTIBODIES IN SCARLET FEVER.

For the demonstration of antibodies in diseases, the germ of which is unknown or incapable of cultivation, two methods may be employed: one rests on the precipitating action, and the other on the complement-binding function, of the anti-serum. Complement deviation offers several advantages over precipitation; these are chiefly:

1. Deviation of the complement occurs on the use of quantities of antigen so small as not to show precipitation.
2. Complement-binding substances appear in the serum at a time when precipitins cannot be demonstrated (Muir and Martin).
3. The complement-binding antibodies are more specific than the precipitins (Bauer-Bruck).

It seemed therefore preferable to use the binding of the complement for the attempt at demonstration of specific antibodies in scarlet fever. Such attempts are not new. In the last few years numerous reports on complement fixation have appeared, instigated by the report of Much and Eichelberg that they obtained fixation of complement with scarlet serum and syphilitic antigen (positive Wassermann reaction). Almost all the following investigations were undertaken with the view only of testing the credibility and specificity of the Wassermann reaction, without consideration of the question as to the presence of specific antibodies in scarlatina.

The fact that some scarlatinal serums give, with some syphilitic extracts, complement deviation, though not impairing the practical value of the Wassermann reaction for syphilis, is of the greatest importance in respect to the use of Bordet's and Gengou's phenomenon as a method of searching for antibodies for an unknown etiologic agent in scarlet fever.

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[1] *Arch. intern. de pharm. et de thérap.*, 1903, 12, p. 205.

The explanation of a positive Wassermann reaction in some cases of scarlet fever may lie in particular properties of the extract of the serum, or in the interaction of both. Thus, for instance, it is obvious that the presence of streptococci, or their soluble substances, in the antigen may give rise to complement deviation with scarlatina serums. This holds for watery organ extracts as well as for alcoholic ones. It was shown by Rüdiger that suspensions of organ extracts have no inhibitory effect on the multiplication of streptococci. As far as alcoholic extracts are concerned, it may be emphasized that our conceptions regarding the insolubility of bacterial antigens in alcohol deserve complete revision. Thus, for instance, Levaditi and Mutermilch[1] have recently proved that those antigenetic constituents of cholera vibrios which are essential for the deviation of complement are soluble in 85 per cent alcohol. What is true for the cholera vibrios must be conceded may be the case of other microorganisms. In the selection of a proper antigen for the investigation of antibodies in scarlet fever this fact must be borne in mind.

Concerning previous work with the aim of finding whether specific antibodies are present in the serum of scarlet-fever patients, with the exception of antibodies for streptococci, the following may be mentioned:

Handel and Schultz,[2] in the second part of their paper, report complement deviation by the serum of scarlet-fever patients on the use of an extract of the liver of a child who had died of a complication of scarlet fever (empyema). With a watery extract they obtained complete complement fixation in 24 cases of 31. The authors emphasize that definite conclusions cannot be drawn from these results with regard to the complement deviation in scarlet fever with homologous extracts. The chief difficulties which arise in estimating the result of these investigators seem to be the following:

1. The antigen (watery extract of the liver) was obtained from a child who had died of an empyema, thus of a secondary infection.
2. No facts as to the etiology of this empyema are given.

Since it possibly was a case of streptococcus infection, the extracts should have been tested with serum of patients suffering from streptococci infection and with streptococcus-immune serum. The positive findings might have been called forth by streptococci or by the related germ causing the empyema.

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[1] *Compt. rend. Soc. de biol.*, 1908, 64, p. 406.

[2] *Ztschr. f. Immunitätsf.*, 1908, 1, p. 91.

Uffenheimer[1] used as antigen the serum of children in the very first stages of the disease and as antibody the serum of convalescents. These experiments proved entirely negative; no inhibition of hemolysis occurred. Uffenheimer, probably rightly, considers the fault of his experiments to lie in too slight concentration of the antigen.

Sommerfeld[2] also used as antigen a watery extract of the liver of scarlet-fever victims. Of 40 serums, 18 reacted positively, 22 negatively. The paper contains no reference regarding the testing of the extract with streptococcus-immune serums.

Hecht, Lateiner, and Wilenko[3] examined 119 scarlet-fever serums for complement fixation, using alcoholic extracts of liver of scarlet-fever patients, all with negative results. They came to the conclusion that no specific properties are present in the scarlet-fever liver.

These investigations, with the exception of Uffenheimer's, are shaped directly after Wassermann's method. So far as the demonstration of Bordet antibodies is concerned, this could be regarded only as an advantage. It is, however, different if we take into consideration the hypothetical basis and the conditions for such experiments. By far the most important of the preliminary questions is the selection of an appropriate antigen.

ANTIGEN.

In the selection of an appropriate antigen it seemed to us a *conditio sine qua non* to choose such organs as seemed to speak clinically as well as anatomically for a place of predilection of the virus. These conditions seem best fulfilled by the kidney, lymph glands, and scales of the skin. We also used liver in order to control the results of previous investigations.

The conditions which an appropriate antigen must fulfil for use in the search for a specific scarlatinal virus seemed to us to be the following:

1. The organs must come from an acute, toxic case of scarlet fever in which the patient succumbed rapidly and without secondary infections and complications.
2. The blood of the patient examined in the last days before death should contain no streptococci.
3. The blood serum ought further, on being tested with an active scarlatinal streptococci emulsion, to show no deviation of the complement. The activity of the streptococcus antigen used should be tested with the corresponding immune serum.

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[1] *Münch. med. Wchnschr.*, 1909, 56, p. 2471.

[2] *Arch. f. Kinderh.*, 1909, 50, p. 38.

[3] *Wien. klin. Wchnschr.*, 1909, 22, p. 523; *Ztschr. f. Immunitätsf.*, 1909, 2, p. 356.

4. The watery organ extracts tested with antistreptococci-immune serum should show no deviation of complement.

From the specially mild character of the scarlet fever in Chicago, it was clear that it was not easy to find such foudroyant toxic cases as answered to these four conditions. We succeeded after long preliminary tests in finding three cases the conditions in which corresponded to all the demands which we had placed on a suitable antigen.

I. *A. S.*—Sick two days and removed to hospital on third. Three years old. No previous diseases. Maximum temperature 104.6.^{c} Great difficulty on swallowing, slight swelling of the cervical lymph glands. Very marked, fiery-red exanthem, relative bradycardia and arrhythmia, delirium, slight albuminuria. Death on the fifth day.

II. *E. W.*—Entered hospital on the second day of the disease. Boy, nine years old. Only previous disease was measles. Maximal temperature 103.8, angina, exanthem, symptoms of cerebral irritation, tachycardia. Death on the fourth day.

III. *H. B.*—Boy, six years old. Entered hospital on the fourth day of the disease. Angina, exanthem, albuminuria, nephritis on the tenth day. Death occurred on the 15th day with uremia.

The organs were removed from the bodies a few hours after death. We used only watery extracts in our experiments, prepared in the following way: The organs were cut up into small pieces with scissors and then with the addition of physiologic salt solution containing 25 per cent phenol rubbed up into a fine emulsion and dried in the vacuum into a hard mass. This was rubbed up into a fine powder and preserved in amounts of 1 gm. in sealed tubes.

For the experiment one-gram powder for every 30 c.c. of physiologic salt solution plus 0.25 per cent phenol was taken. The mixture was shaken for two hours in the shaking apparatus and then placed in a brown bottle in the icebox. After 48 hours the extract is ready for use. The required amount is rendered clear by centrifugation immediately before use.

The kidneys, lymph glands, and liver were treated in this way.

The scales were obtained from a large number of cases. In order to macerate them completely, we allowed them to stand in a 15 per cent antiformin solution for 72 hours. The excess of alkali was removed by the addition of 5 per cent sulfuric acid, drop-wise, and of chlorine by a 5 per cent sodium-hyposulfite solution.

The streptococcus emulsion which was used as control was prepared from blood-agar cultures of streptococci obtained by blood culture from scarlet fever, and suspended in physiological salt solution, the growth of one tube to about 2 c.c. salt solution. Before use this emulsion was tested for its inhibiting and hemolytic effect and that amount employed as a test of the serum of moribund cases, which gave complete hemolysis with the system. For instance:

Streptococcus Emulsion	Physiologic Salt Solution	Complement	Amboceptor (3 × Hemolytic Dose)	Blood	Result
1.0 c.c.	1.0 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	No hemolysis
.5 c.c.	1.5 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	Hemolysis
.4 c.c.	1.6 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	"
.3 c.c.	1.7 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	Slight hemolysis
.2 c.c.	1.8 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	" "
.15 c.c.	1.85 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	Complete "
.1 c.c.	1.9 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	" "
.05 c.c.	1.95 c.c.	.1 in 1 c.c.	1 c.c.	1 c.c.	" "

The extracts were used in amounts of 15 per cent in testing the scarlet-fever serum. Only the scarlatinal streptococcus emulsions that gave complete inhibition with streptococcus-immune serums were used.

ANTISERUMS.

The serums of scarlet-fever patients were obtained in all periods of the disease, from the fourth day up to the 102d day. The blood was collected sterile by puncture of the vein of the arm. After coagulation was complete, the serum was pipetted off, centrifugated, and when entirely clear, heated at 56° for 30 minutes. We employed only inactivated serum, which was obtained on the day of the experiment.

COMPLEMENT.

Fresh guinea-pig serum was used as complement. It was titrated exactly against the hemolytic system and twice the multiple of the dose of complement was used, which just caused complete hemolysis.

HEMOLYSIN.

As amboceptor was used the serum of rabbits immunized against sheep's blood. In order to know whether normal amboceptor for sheep's blood was contained in the human serum, we tested this every time without the addition of antigen and amboceptor (see Tube No. 4 in Example 2). If by this control test we obtained hemolysis, we removed the natural sheep amboceptor by absorption and then repeated the experiment. The procedure employed was as follows: 3 c.c. inactivated serum and 1 c.c. sheep's blood corpuscles, washed three times, were allowed to remain in contact for one-half hour in the icebox. Then they were centrifugated, and the reaction was carried on with the serum, which was pipetted off.[1]

BLOOD.

The blood was a 5 per cent suspension of sheep corpuscles in physiologic salt solution.

CONTROLS.

Each extract was tested: (1) with antistreptococcus-immune serum, (2) for hemolytic (hemotoxic) action, (3) for anticomplementary action, (4) with normal serum, and (5) with serums of other diseases, such as measles, erysipelas, nephritis, etc.

The normal extracts which served as controls were tested in the same way.

Each serum was tested: (1) for anticomplementary action (without antigen), (2) for hemolytic action without antigen and without amboceptor, and (3) with normal extract.

After titration of the amboceptor and complement and examination of the organ extracts, the chief experiment was made in the following way:

EXAMPLE OF EXPERIMENT.

Tube	Extract	Serum	Complement	Amboceptor	Blood	Result
1 . . .	.2 lymph gland scarlet	.2 scarlet	1 c.c.	1 c.c.	1 c.c.	No hemolysis
2 . . .	1.1 “ “ “	.1 “	1 c.c.	1 c.c.	1 c.c.	“ “
3 . . .	. . . .	.2 “	1 c.c.	1 c.c.	1 c.c.	Complete “
4 . . .	. . . .	.2 “	1 c.c.	. . . . .	1 c.c.	No “
5 . . .	.4 lymph gland	. . . .	1 c.c.	1 c.c.	1 c.c.	Complete “
6 . . .	.2 “ “	. . . .	1 c.c.	1 c.c.	1 c.c.	“ “
7 . . .	.2 “ “	.2 normal	1 c.c.	1 c.c.	1 c.c.	“ “
8 . . .	.1 “ “	.1 “	1 c.c.	1 c.c.	1 c.c.	“ “
9 . . .	.4 normal lymph gland	. . . .	1 c.c.	1 c.c.	1 c.c.	“ “
10 . . .	.2 “ “ “	. . . .	1 c.c.	1 c.c.	1 c.c.	“ “
11 . . .	.2 “ “ “	.2 scarlet	1 c.c.	1 c.c.	1 c.c.	“ “
12 . . .	.1 “ “ “	.1 “	1 c.c.	1 c.c.	1 c.c.	“ “
13 . . .	.2 “ “ “	.2 normal	1 c.c.	1 c.c.	1 c.c.	“ “
14 . . .	.1 “ “ “	.1 “	1 c.c.	1 c.c.	1 c.c.	“ “
15 . . .	. . . .	. . . .	1 c.c.	1 c.c.	1 c.c.	“ “
16 . . .	. . . .	. . . .	1 c.c.	1 c.c.	1 c.c.	No “
17 . . .	. . . .	. . . .	. . . . .	. . . . .	1 c.c.	“ “

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[1] We have used this control for more than one year in our routine work with the Wassermann reaction and can recommend it most warmly, since negative reactions with surely syphilitic serum can be avoided in this way.

TABLE 1.

Case	Name of Patient	Age in Years	Day of Sickness when Blood Was Examined	Kidney Extract	Liver Extract
1	M.B.	10	3	−	−
2	D.G.	8	6	−	−
3	H.B.	7	14	+	+
4	J.C.	9	11	+	+
5	N.L.	10	14	+	+
6	S.F.	1.5	7	−	−
7	S.L.	8	15	+	−
8	L.E.	5	16	+	−
9	W.G.	16	19	+	+
10	B.	7	8	−	−
11	L.M.	8	12	+	+
12	L.P.	12	13	+	+
13	C.G.	12	12	+	−
14	B.F.	2.5	11	−	−
15	D.A.	16	4	−	−
16	W.B.	3	7	−	−
17	W.L.	3	18	+	+
18	B.K.	24	13	+	−
19	S.E.	5	9	+	−
20	K.O.	5	13	+	+
21	D.V.	5	8	−	−
22	D.R.	10	8	−	−
23	G.R.	15	13	+	+
24	R.R.	5	19	+	+
25	W.E.	49	16	+	−
26	B.M.	3	5	−	−
27	B.A.	4	7	−	−
28	S.M.	6	19	+	+
	S.M.	6	42	+	+
29	A.H.	7	14	+	+
30	D.M.	8	5	−	−
31	J.R.	7	9	−	−
32	C.B.	3	17	+	+
33	A.M.	22	11	+	−
34	L.C.	18	16	+	−
35	V.H.	3	9	−	−
36	B.J.	5.5	13	−	−
37	C.E.	16	14	+	−
38	W.B.	17	19	+	+
39	R.G.	4	21	+	+
40	B.J.	16	5	−	−
41	K.M.	5	11	−	−
42	M.K.	5	14	+	+
43	B.M.	4	9	+	−
44	B.D.	5	10	−	+
45	L.W.	21	3	−	+
46	K.E.	15	12	+	+
47	R.R.	8	19	+	−
48	S.H.	4	16	+	+
49	C.M.	8	5	−	−
50	G.J.	12	10	−	−

In this way each scarlet serum was tested with the various organ extracts.

Beside normal serum, serums of measles, diphtheria, erysipelas, malignant tumors, tuberculosis, and nephritis were used as controls. None of these control serums prevented hemolysis with the extract.

We first examined 50 cases with liver and kidney extracts. The results are given in Table 1. Further, 25 cases with lymph glands, kidney and liver extracts (Table 2). Since the extract of lymph

TABLE 2.

Case	Name of Patient	Age	Day of Sickness when Blood Was Examined	Lymph Gland Extract I	Kidney Extract	Liver Extract
51	P.L.	20	11	+	–	–
52	H.E.	7	15	+	+	+
53	C.A.	15	9	+	–	–
54	K.E.	30	13	+	+	–
55	R.S.	8	12	+	–	–
56	S.R.	4.50	8	–	–	–
57	L.C.	25	15	+	+	+
58	P.S.	7	9	–	–	–
59	K.A.	6	17	+	+	–
60	R.R.*	1.66⅔	19	+	+	+
61	G.M.	2.50	8	–	–	–
62	B.B.	18	12	+	+	–
63	H.L.	30	7	–	–	–
64	D.M.	28	11	+	+	–
65	L.F.	12	15	+	+	+
66	L.M.B.	28	9	+	–	–
67	J.A.	24	11	+	+	+
68	B.S.	7	14	+	+	–
69	R.R.	11	13	+	+	–
70	E.H.	23	15	–	+	+
71	M.A.	24	14	+	–	–
72	W.C.	8	19	–	+	+
73	S.L.	9	11	+	–	–
74	B.G.	6	15	–	+	–
75	W.V.	4	13		–	–

* Had scarlet fever twice in one year.

+ Designates inhibition of hemolysis.

– Designates hemolysis.

glands proved the most effective, far more so than all the others, we limited our further work to experiment with this extract alone (Table 3).

The extract prepared of the scales proved negative in all instances and therefore no reference is given to this work here.

If now the positive reactions recorded in the three tables are compared, we see that:

Of 75 cases, 43 were positive with kidney extract, 57.3 per cent.
Of 75 “ 25 “ “ “ liver “ 32 “
Of 118 “ 81 “ “ “ lymph-gland extract, 68.6 per cent.

Thus the extract prepared from the cervical lymph glands proved the most powerful antigen.

An extract prepared from a normal kidney of a scarlet-fever case and from one of a case of scarlet-fever nephritis showed no difference in activity.

It is striking that of the serums examined of cases of nephritis scarlet fever, all gave a positive reaction in each case.

TABLE 3.

Case	Name of Patient	Age	Day of Sickness When Blood Was Examined	Lymph Gland Extract II
76	A.R.	7	11	+
77	D.V.	6	15	+
78	F.R.	5	9	−
79	F.M.	7	12	+
	F.M.	7	58	+
80	P.M.	16	17	+
81	K.A.	10	12	+
	K.A.	19	31	+
82	W.F.	9	4	−
83	B.A.	8	17	+
84	T.N.	20	13	+
85	L.F.	9	16	+
86	M.H.	12	29	+
87	B.R.	22	14	+
	B.R.	22	46	+
88	E.A.	9	12	−
89	Z.J.	7	5	−
90	M.K.	18	8	−
91	M.M.	20	15	+
	M.M.	20	39	+
92	S.T.	29	4	−
93	F.I.	22	15	+
94	N.O.	21	11	+
95	H.R	21	15	+
96	S.E.	28	14	+
97	K.A.	5	24	+
	K.A.	5	63	+
98	E.R.	15	21	+
99	T.C.	23	12	−
100	M.G.	15	13	−
101	G.W.	12	14	+
102	I.I.	6.5	17	+
	I.I.	6.5	84	+
103	W.E.	9	4	+
104	I.I.	6.5	20	+
105	G.P.	4	15	+
106	L.S.	9	13	+
107	C.A.*	27	12	−
108	W.C.	5	17	+
	W.C.	5	46	+
109	D.W.	2	14	−
110	A.R.	28	19	+
	A.R.	28	102	+
111	F.G.	16	28	+
112	E.H.	18	21	+
113	S.G.	3	16	−
114	G.A.	28	13	−
115	B.M.	5	17	+
116	P.E.	22	18	+
	P.E.	22	48	+
117	L.S.	12	15	+
118	S.F.	5	12	−
119	S.E.	7	12	+
120	S.G.	4	4	−
121	W.A.	12	15	+
	W.A.	12	35	+
122	F.M.	6	13	+
123	F.D.	7	14	+
124	F.S.	7	14	+
	F.S.	7	45	+
125	H.M.	12	19	−
126	S.M.	22	12	−
127	H.O.	11	10	+
128	D.E.	7	15	+

* Designates nephritis.

+ Designates inhibition of hemolysis.

− Designates hemolysis.

TABLE 3.—*Continued.*

Case	Name of Patient	Age	Day of Sickness When Blood Was Examined	Lymph Gland Extract II
129	M.H.	9	17	+
130	W.M.	6	13	+
131	D.C.	19	11	—
132	F.H.	12	16	+
133	H.R.	6	13	—
134	P.E.	6	15	+
135	F.F.	8	18	+
136	S.M.	4.5	12	+
137	J.E.	4	11	—
138	M.A.	7	24	+
	M.A.	7	69	+
139	M.J.	12	20	+
140	M.A.	10	21	+
141	T.M.	8	11	+
142	McC.J.	27	14	+
143	W.M.	26	15	—
144	L.E.	16	13	+
145	L.J.	11	24	+
146	S.F.	13	21	+
147	T.L.	8	19	—
148	O'B.J.	10	17	+
149	L.J.	22	14	—
150	L.L.	17	24	+
151	C.M.	29	19	+
152	A.R.	4	16	—
153	W.M.	23	27	+
154	C.F.	5	23	+
155	D.G.	17	21	+
156	M.B.	8	18	+
157	W.S.	4	24	—
158	W.F.	14	12	+
159	H.R.	5	14	+
160	M.E.	22	17	—
161	S.L.	12	25	+
	S.L.	12	38	+
162	B.L.	9	21	+
163	M.L.	3	18	—
164	F.G.	13	17	—
165	V.G.F.	17	19	+
	V.G.F.	17	34	+
166	V.G.W.	15	20	+
167	L.F.	11	17	+

* Designates nephritis.
+ Designates inhibition of hemolysis.
— Designates hemolysis.

In respect to the time of first appearance of the antibodies, it may be said that they do not seem to be present in the blood in demonstrable quantities before the onset of the second week, the eighth day being the earliest in which positive reaction was obtained. So far as the duration of the action is concerned, the serum was found to be active in the 12th week, and in one case of scarlet nephritis even in the 16th week.

The activity of the extracts gradually diminishes; when in March our work was interrupted, to be begun again in May, the lymph-gland extract formerly so active had lost its antigenetic properties, and a new extract (II) had to be prepared.

The following conclusions would seem to be suggested by the results obtained:

1. The serum of scarlet-fever patients contains specific antibodies for an unknown virus.

2. This unknown virus seems to be present especially in the cervical lymph glands.